

W-PM-Sym-1

HOW SYNTHETIC CHELATORS ACHIEVE SELECTIVITY BETWEEN METAL IONS. ((Roger Y. Tsien)) Univ. of California-San Diego.

W-PM-Sym-2

TUNING THE ION SELECTIVITY AND KINETICS OF EF-HAND CALCIUM SIGNALING PROTEINS. (Joseph J. Falke)
Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215.

The EF-hand calcium binding motif is found widely in calcium-regulated signaling pathways. Currently over 1000 examples of the EF-hand have been identified in the protein sequence data base, making this among the most prevalent binding motifs in nature. The heart of the EF-hand site is its 12-residue calcium binding loop, which possesses a conserved framework but also exhibits considerable sequence variability. Such variability plays an important functional role by tuning the ion binding parameters of the motif, thereby enabling calcium binding affinity, specificity and kinetics to be optimized for the activation of different signaling pathways. Systematic engineering of the EF-loop in prokaryotic and eukaryotic model sites has begun to reveal the structural and electrostatic mechanisms underlying the ion charge and size selectivities of the motif, and the basis of kinetic tuning. Another important type of tuning is provided by intermolecular interactions between an EF-hand protein and its effector protein(s). The range of ion binding parameters produced by such intermolecular tuning has been investigated by systematic studies of calmodulin binding to different target helices. The results of these and other studies will be discussed.

W-PM-Sym-3

Ca CHANNEL SELECTIVITY. ((Richard W. Tsien)) Stanford Univ.

W-PM-Sym-4

IONIC SELECTIVITY IN CYCLIC NUCLEOTIDE-GATED CHANNELS.
((R. MacKinnon)) Harvard Med. Sch.

MACROMOLECULAR PACKING**W-PM-A1**

K⁺-INDUCED SUPRAMOLECULAR ASSEMBLY OF G-QUADRUPLEXES IN d(CGG)₄. ((Fu-Ming Chen)) Department of Chemistry, Tennessee State University, Nashville, TN 37209-1561.

A remarkable circular dichroic (CD) intensity enhancement of nearly two orders of magnitude is induced in d(CGG)₄ by molar [K⁺] to result in ψ -type spectral characteristics and aggregate formation. The kinetics of this transformation are extremely slow at pH 8 but are found to be greatly facilitated in acidic conditions. Kinetic profiles via absorbance or ellipticity monitoring resemble those of autocatalytic reacting systems with induction periods. More than 0.8 M KCl is needed to observe the onset of aggregation within the time span of 24 hrs at 20 °C and pH 5.4. CD spectral evidence suggests the formation of parallel G-tetraplexes prior to the onset of aggregation. Although both d(TGG)₄ and d(CGG)₄T appear to form parallel G-quadruplexes, they fail to exhibit the aggregation phenomenon, implicating the crucial roles played by the terminal G and base protonation of cytosines in the observed process. A plausible mechanism for the formation of a novel self-assembled structure is speculated: Aided by the C⁺•C base pair formation, parallel quadruplexes are initially formed and subsequently converted to quadruplexes with contiguous G-tetrads and looped-out cytosines due to high [K⁺]. These quadruplexes then vertically stack as well as horizontally expand via inter-quadruplex C⁺•C base pairing to result in dendrimer-type of self-assembled super structures.

W-PM-A2

THE MEASUREMENT OF CHARGE ON DNA BY EQUILIBRIUM ELECTROPHORESIS. ((T.M. Laue, T.M. Ridgeway, J.O. Wooll, D.A. Stephenson, J.B. Chaires, D.B. Hayes and D.M. Green)) Dept. of Biochemistry and Molecular Biology, University of New Hampshire, Durham, NH 03824.

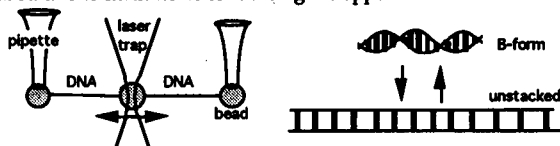
The net charge on DNA is inextricably linked to its structure and binding interactions. Equilibrium electrophoresis is a method by which the net charge on a molecule in solution can be obtained. The DNA solution being examined forms a steady-state concentration gradient in which the flux due to electrophoresis is exactly counter-balanced by the flux due to diffusion. The resulting concentration gradient is described by $c(x) = c_0 e^{\sigma x}$. The net charge, Q , is determined from the exponent, $\sigma = QE/k_B T$, where E is the electric field, k_B is Boltzmann's constant and T is the temperature. Experiments using defined oligonucleotides demonstrate that the measured charge, Q , is the same as that predicted by the polyelectrolyte theory. Different configurations of the far larger plasmid DNA structures have been examined. While the charge on relaxed-circular DNA is readily measured, both the linear and supercoiled DNA forms do not establish stable gradients under ordinary operating conditions. Initial studies seem to indicate that the electric field surrounding the DNA is altered with the different plasmid configurations. Experimental approaches to exploring and manipulating this phenomena will be described. Funded by NSF DIR 8914571 and BIR 9314040.

W-PM-A3

STRETCHING DNA BEYOND ITS B-FORM CONTOUR LENGTH

(Steven B. Smith, Yujia Cui, Andrew C. Hausrath and Carlos Bustamante) Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

DNA molecules under longitudinal stress can unwind and extend into a stable form with 6.3 Å rise/bp. In our experiment, single molecules of dsDNA from λ -phage (48.5 kbp) were attached to 2 μ m polystyrene beads and stretched between glass micropipettes in 1 M NaCl buffer. Using a central "reporter" bead, it was seen that DNA could be over-stretched to 185% of its B-form contour length before breaking. Unstacking initiated suddenly at ~50 pN force, but above this amount, only a small additional force was needed to finish unstacking all the bases in a λ -phage DNA molecule. With the pipettes separated by 150% of the DNA's B-form length and ~50 pN tension in the chains, a laser beam trap, capable of exerting only 1 pN force on the reporter bead, could move that bead back and forth so as to completely unstack one chain while condensing the opposite chain into B-form.



W-PM-A5

STRUCTURE AND DYNAMICS OF THREE-WAY DNA JUNCTIONS.

((Y.L. Lyubchenko¹, L.S. Shlyakhtenko^{1,2}, E. Appella³ and I.V. Kutyavin⁴)) ¹Dept. of Microbiology, Arizona State University, Tempe, AZ 85287-2701, ²SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025, ³NCI, NIH, Bethesda, MD 20892, ⁴Microprobe Corp., Bothell, WA 98021. (Spon. by J. Allen)

Tertiary structure of immobile three-way junctions was investigated by cyclization method coupled with separation of linear and circular ligated oligomers by two-dimensional gel electrophoresis. Junctions with perfect pairing at the branch point adopt non-flat pyramidal geometry. Broad distribution of circular molecules on their length indicates high mobility of the arms of junctions. Mismatches at the branch point decrease this mobility. Effect depends on the position of arms relative to the position of the mismatch. GG-mismatch at the hairpin arm almost freezes a very acute angle between two linear arms. Bulges at the branch point induce transition of pyramidal geometry of junction into T-like geometry. This type of geometry is characterized by rather high mobility of arms. The character of the mobility of three-way junctions will be discussed.

W-PM-A7

AUTOMATIC IDENTIFICATION OF HYDROPHOBIC CLUSTERS.

((M. H. Zehfus)) Division of Medicinal Chemistry and Department of Biochemistry, The Ohio State University, Columbus, OH 43210.

An objective method is proposed to identify hydrophobic clusters in proteins of known structure. Hydrophobic clusters are discovered by locating groups of side chains that are locally compact. Small hydrophobic clusters contain more than 65% hydrophobic residues, are located in a protein's interior, and contain few main chain atoms. Larger hydrophobic clusters range between 40 and 65 % hydrophobic residues, contain some residues from the protein's surface, and encompass main chain atoms as large pieces of regular secondary structure.

Good correlation is seen between hydrophobic clusters and experimental evidence on early folding intermediates when the clusters are filtered to remove units with less than nine hydrophobic residues. This confirms the hypothesized link between hydrophobic regions and early folding intermediates, and suggests that hydrophobic regions must have some minimum size before they confer enough stability to be functionally useful.

W-PM-A4

STRUCTURE and DYNAMICS of DNA TRIPLET REPEATS, CTG/CAG

S. V. Santhana Mariappan, Goutam Gupta, and A. E. Garcia, Theoretical Biology and Biophysics, T-10, MS-K710, Los Alamos National Laboratory, Los Alamos, NM 87545

Huntington's Disease and Myotonic Dystrophy are linked to expansion of the DNA triplet repeats, CTG/CAG. It is our hypothesis that the formation of self-assembled hairpin structures causes active expansion of these repeats. The nature of the hairpin structure depends on the repeat number, n . The hairpins with odd n have 3 nucleotides in the loop whereas the hairpins with even n have 4 nucleotides in the loop (Figure 1). One- and two-dimensional ¹H and ³¹P NMR and restrained molecular dynamics studies are performed on (CTG)_{5,6} and (CAG)_{5,6} for deriving detailed structures of these hairpins. Base-pair open-closure dynamics and site-specific mobility in these structures are derived from base-catalyzed proton exchange, (¹H-¹H) cross-relaxation, and ³¹P-relaxation. The order parameters computed from the MD trajectories are compared with those obtained from experiments.

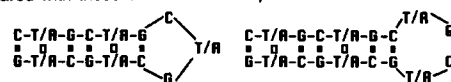


Figure 1. Base-pairing and folding patterns of (CTG)_n and (CAG)_n for $n=5$ (left) and $n=6$ (right). Note the presence of T.T or A.A mismatches in the stem.

W-PM-A6

INVESTIGATING PROTEIN FOLDING VIA SIMILAR ARCHITECTURAL MOTIFS IN PROTEIN CORES AND IN SUBUNIT INTERFACES ((S.L. Lin, C-J. Tsai, R. Nussinov)) Laboratory of Mathematical Biology, NCI-FCRF, Frederick, MD 21702.

Supersecondary architectural motifs found in protein cores have two interesting features: (1) Recurring in unrelated proteins, they indicate a certain degree of sequence independence of the structural organization of polypeptide chains. (2) Composed of segments not necessarily consecutive in sequence, they represent the onset of regularities of higher architectural design beyond that of the secondary structures. These features raise the possibility that the motifs can also be significant in the organization of the subunit interfaces of multi-chain proteins, in spite of a pathway different from that of the chain folding. A study of these proteins has uncovered a rich variety of motifs in the interfaces. The general characteristics of them suggest that the same physical principles govern the association of subunits and the folding of protein cores. The detailed differences in the motifs between their appearances in the cores and in the interfaces suggest that either the subunits can be associated in conformations far from global minimum, or these differences have recorded the footprints of the pathway leading to protein cores and interfaces.

W-PM-A8

EXHAUSTIVE ENUMERATION OF PROTEIN CONFORMATIONS USING EXPERIMENTAL RESTRAINTS. ((R.S. DeWitte S.W. Michnick, and E.I. Shakhnovich)) Chemistry Department, Harvard University, Cambridge MA. 02138.

We present an efficient new algorithm which enumerates *all* possible conformations of a protein which satisfy a given set of distance restraints. Rapid growth of all possible self-avoiding conformations on the diamond lattice provides construction of α -carbon representations of a protein fold. We investigated the dependence of the number of conformations on pairwise distance restraints for the proteins crambin, pancreatic trypsin inhibitor, and ubiquitin. Knowledge of between one and two contacts per monomer is shown to be sufficient to restrict the number of candidate structures to approximately 1000 conformations. Pairwise root mean squared deviations (RMSD) of atomic position comparisons between pairs of these 1000 structures revealed that these conformations can be grouped into at most 25 families of structures. These results suggest a new approach to assessing alternative protein folds given a very limited number of distance restraints. Such restraints are available from several experimental techniques such as NMR Nuclear Overhauser Effect Spectroscopy (NOESY), Energy Transfer Fluorescence Spectroscopy, and Cross Linking experiments. This work focuses on exhaustive enumeration of protein structures with emphasis on the possible use of NOESY-determined distance restraints.

W-PM-A9

Multi-basin Dynamics of a Protein in Aqueous Solution. ((Angel E. Garcia)) Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM 87545

We will show that non-linear motions describing oscillations around multi-centered distributions are responsible for most of the atomic fluctuations sampled by a protein on a 100 picoseconds time-scale. These atomic fluctuations are not well described by large fluctuations of individual atoms or small group of atoms, but by concerted motions of many atoms. These modes are non-linear in the sense that they describe transitions among different basins of attraction. The signature of these non-linear modes can be seen in various local and global structural variables. A method for extracting molecule optimal dynamic coordinates (MODC) will be presented. A generalization of this method to identify small (1-3) dimensional subspaces of the configurational space will be used to conclusively show a description of the protein dynamics within the context of multi-basin dynamics.

K⁺ CHANNEL ELECTROPHYSIOLOGY

W-PM-B1

ATP-SENSITIVE K⁺ CHANNELS IN SMOOTH MUSCLE OF CEREBRAL ARTERIES: PHARMACOLOGICAL AND HORMONAL MODULATION. (T. Kleppisch and M. T. Nelson) Univ. Vermont, Dept. Pharmacol., Colchester, VT 05446.

Synthetic activators of K_{ATP} channels (e.g. pinacidil [PIN], lemakalim [LEM]), calcitonin gene-related peptide (CGRP) and hypoxia cause dilations of cerebral arteries that are attenuated by the blocker of K_{ATP} channels glibenclamide (GLIB). Here, we provide the first characterization of K_{ATP} channels in smooth muscle cells isolated from rabbit basilar arteries. PIN (10 μM) and LEM (10 μM) induced GLIB-sensitive whole-cell currents of -48.4 ± 4.6 pA (n=38) and -44.2 ± 6.8 pA (n=20) at -60 mV with 140 mM external K⁺ when the cells were dialyzed with 0.1 mM ATP and 140 mM K⁺. PIN-induced GLIB-sensitive currents were not voltage-dependent and were K⁺ selective: the whole-cell current-voltage relationships for currents activated by PIN were essentially linear and the reversal potential followed closely the theoretical equilibrium potential for K⁺ with 6, 60 and 140 mM external K⁺ (intracellular K⁺ 140 mM). GLIB-sensitive currents in the presence of PIN were inhibited by intracellular ATP: increasing pipette ATP from 0.1 to 3 mM reduced these currents 3-fold to -16.1 ± 4.9 pA (n=7). PIN-induced GLIB-sensitive currents in intact cells, measured with the perforated patch technique, were similar to those in cells dialyzed with 3 mM ATP (-22.2 ± 5.2 pA, n=8). The blockers of Ca²⁺-activated K⁺ channels, iberiotoxin and charybdotoxin, did not affect PIN-induced inward currents (n=4), whereas tetraethylammonium blocked the current by 36.3 ± 1.8% (1 mM, n=5) and 79.8 ± 4.3% (10 mM, n=5). PIN-induced currents were also blocked by 4-aminopyridine (10 mM, 78.9 ± 6.6%, n=3). Moreover, the vasoconstrictors serotonin (10 μM) and histamine (10 μM) inhibited PIN-induced currents by 51.5 ± 7.0% (n=5) and 81.3 ± 6.7% (n=7) respectively. The vasodilators adenosine (5 μM) and CGRP (20 nM), in contrast, increased K_{ATP} currents by -28.5 ± 5.5 pA and -19.7 ± 5.7 pA. We conclude that smooth muscle cells from cerebral arteries have K_{ATP} channels and that these channels are modulated by endogenous vasoconstrictors and vasodilators.

Supported by the NIH and the Alexander von Humboldt-Stiftung

W-PM-B3

G-protein Subunit Activation of Cardiac K channels During Cholinergic Stimulation. ((Jinliang Sui, Kim W. Chan, Ravi Iyengar and Diomedes E. Logothetis)) Mount Sinai School of Medicine, CUNY, New York, NY.

Purified G-protein subunits (G_{βγ} and activated G_α^{*}, G_α-GTPγS, subunits) have been shown to activate cardiac K channels. Lack of a specific inhibitor of channel activation by one but not the other G-protein subunit has precluded elucidation of their relative role during cholinergic stimulation. We have examined the effects of a peptide (QEHA) encoding amino acids 956-982 of adenyl cyclase 2 (AC2) on G_{βγ} activation of the K channel in chick embryonic atrial cells. QEHA blocks G_{βγ} effects on AC2, AC1, PLCβ3, and βARK. 50 μM QEHA also blocked 98% of G_{βγ} activation of atrial K channels. We tested the effects of QEHA peptide on GTPγS activated K channels. In most patches the peptide blocked 90-100% of the GTPγS stimulated K channel activity. Similarly, when the peptide was applied together with GTP, it blocked K channel activation. We are currently testing the effects of QEHA peptide on G_α^{*} stimulated K channel activity. Our results suggest that G_{βγ} is the predominant G-protein subunit used during cholinergic stimulation. Using probes spanning the M1 through the M2 region of a cloned G-protein-gated channel (Kubo *et al.*, 1993; Dascal *et al.*, 1993), we screened a human brain cDNA library. A human homologue was isolated (hbGIRK1) which showed differences in 6 amino acid positions from the published rat cardiac and brain channels. Functional coexpression of this recombinant channel and the human muscarinic 2 receptor in *Xenopus* oocytes gave inward rectifying currents sensitive to 200 μM BaCl₂. rcGIRK1 channel was recently reported to be activated by G_{βγ} but not G_α^{*} subunits. We are currently testing the effects of the QEHA peptide on the cholinergic activation of hbGIRK1.

W-PM-B2

INFLUENCE OF Na⁺-K⁺-PUMP ACTION ON THE ACTIVITY OF K_{ATP}-CHANNELS IN ISOLATED GUINEA PIG HEART CELLS. ((K. Berndorf*, L. Priebe and M. Friedrich)) Zentrum für Physiologie, Universität zu Köln, Germany. *Heisenberg-fellow of the DFG.

Ionic current through ATP-regulated K⁺ channels (I_{KATP}) was studied with a patch clamp technique in ventricular heart cells of the guinea pig under conditions of metabolic poisoning with the uncoupler 2,4-dinitrophenol (DNP). Maintained exposure of the cells to DNP caused transient appearance of whole cell I_{KATP}. At the time when I_{KATP} reached several nA, block of the forwardly running Na⁺-K⁺-pump with 0.5 mM strophanthidin caused an exponential decay of I_{KATP} (τ = 4.51 ± 1.89 s; n=8). This time course was significantly slower than the respective decay of the current generated by the Na⁺-K⁺-pump itself (τ = 0.28 ± 0.03 s; n=5). Hyperpolarization for 2 s from 0 mV to -100 mV and -150 mV decreased I_{KATP} at 0 mV by 34.2 ± 14.1% (n=8) and 37.6 ± 9.4% (n=8), respectively. I_{KATP} at 0 mV relaxed subsequently to the larger initial I_{KATP} level in an approximately exponential manner. Forcing the pump to run backwardly by removing extracellular K⁺ caused permanent disappearance of I_{KATP}. In the absence of external K⁺ ions, both application of strophanthidin and washing it out induced transient performance of I_{KATP}. Strophanthidin did not affect I_{KATP} in the absence of any pump action (Na⁺-K⁺-free Tyrode in the bath). In cell-attached patches, strophanthidin applied to the bath caused a decay of I_{KATP} with a similar time course as in whole cell experiments. The data support that all manipulations at the pump influence the cytosolic [ATP] and thus the activity of the K_{ATP}-channels. [ATP] at the cytosolic face of the membrane may drop to practically zero, thereby passing an "ATP-window" in which the channels first open and subsequently close. The spatial dimension of the volume in which this modification of [ATP] takes place is at least several micrometers.

W-PM-B4

CELLULAR ELECTROPHYSIOLOGY AND IMMUNOCYTOCHEMISTRY OF SINGLE ISOLATED MURINE EMBRYONIC CARDIAC MYOCYTES: A NEW METHOD TO STUDY CARDIOGENESIS IN VIVO AND IN VITRO. ((P.A. Doevendans, M. Davies, S. Kubalak, R.H. An, R. Bangalore, L. Rubin, R.S. Kass, and K.R. Chien)) Dept. of Medicine, U.C. San Diego, San Diego, CA 92093-0613, and Dept. of Physiology, U. of Rochester, Rochester, NY 14642. (Sponc. by V.K. Sharma)

We have used electrophysiological and immunocytochemical techniques to test the validity of the mouse embryoid body (EB) system as an *in vitro* model for early cardiogenesis. Cardiomyocytes, isolated and purified from differentiated EB's (day 14), served as our *in vitro* model. We also studied atrial and ventricular cells isolated and plated from mouse embryos (day 11-14) as an *in vivo* model. Cells in both models were studied first with conventional and perforated patch whole cell patch clamp procedures, and then, after fixation, identified by immunocytochemistry. Polyclonal antibodies were used directed specifically against either atrial (MLC 2a) or ventricular (MLC 2v) myosin light chain 2. A monoclonal antibody, α-myomesin was used as a control in all cells to detect the presence of myofibrils. In the EB's myocytes could be identified by positive staining for α-myomesin: co-staining with MLC 2a was positive in 98% of the cells, compared with 39% for MLC 2v. The EB cells expressed voltage-gated Na⁺, (L-type) Ca²⁺, and inactivating but no inwardly rectifying K⁺ channel currents. No distinction could be made between atrial and ventricular cellular electrophysiology. The ventricular and atrial cells *in vivo*, expressed a similar set of ion channels. L-channel currents *in vivo* were insensitive both to isoproterenol and CPT-cAMP, a membrane permeable cAMP analog as were preliminary recordings of L channel modulation *in vitro*. We conclude that in day 14 EB derived cardiomyocytes ion channels are not yet dependent on ventricular specification, are similar to channels expressed in the *in vivo* (day 11-14) setting. Our results demonstrate that the EB system is a valid and useful system for studies of cardiogenesis and, particularly, for developmentally-related changes in cardiac ion channels and their regulation by neurohormones.

W-PM-B5

A NOVEL K-CONDUCTANCE MAY DETERMINE THE RESTING POTENTIAL IN RABBIT PULMONARY ARTERIAL MYOCYTES. ((A.M. Evans, O.N. Osipenko and A.M. Gurney)) Dept. of Pharmacology, UMDS, St. Thomas' Hospital, London SE1 7EH, U.K. (Spon. by N.B. Standen)

Recent reports have argued a role for a variety of K-currents in setting the resting membrane potential (RMP) of vascular smooth muscle cells. These include the ATP-sensitive K-current (I_{KATP}), the Ca²⁺-activated K-current (I_{KCa}) and the delayed rectifier current (I_{KDR}). Using whole-cell, voltage- and current-clamp techniques, we have studied the relative contribution of these currents to RMP (measured as zero current potential) in rabbit pulmonary arterial myocytes. Blockade of I_{KCa} and I_{KATP} by extracellular tetraethylammonium (10 mM) and glibenclamide (10 μ M) had little effect on the RMP, which remained at -47.7 ± 0.6 mV (mean \pm s.e.m., $n=45$). With these drugs present, and inactivation of I_{KDR} and the A-current induced by prolonged (up to 30 min) depolarization to 0 mV, an RMP of -45 ± 1 mV ($n=14$) was still measured immediately on switching to I=0, without recovery of the inactivated channels. Under these conditions, a non-inactivating current remained, which had a threshold for activation more negative than -50 mV. Also, 4-aminopyridine (10 mM) partially blocked the non-inactivating current and depolarized the RMP to -22 ± 2 mV ($n=12$). The reversal potential of this current varied with the extracellular K⁺ concentration as predicted for a K-conductance. Furthermore substitution of intracellular Cs⁺ for K⁺ abolished both this current and the RMP (-0.4 ± 1.0 mV, $n=5$). Our data suggest that a novel K-conductance, which we denote I_{KRP} , determines the resting membrane potential in rabbit pulmonary artery myocytes. (Supported by the British Heart Foundation.)

W-PM-B7

TWO COMPONENTS OF THE DELAYED RECTIFIER K⁺ CURRENT IN VENTRICULAR MYOCYTES: THEORETICAL FORMULATION AND THEIR ROLE IN REPOLARIZATION ((J. Zeng and Y. Rudy)) Dept. of Biomedical Engineering, Case Western Reserve Univ., Cleveland, OH 44106. (Spon. by grant HL-49054 from NIH)

Two distinct delayed rectifier K⁺ currents, I_{Kr} and I_{Ks} , were found recently in ventricular cells. We formulated these currents theoretically and investigated their roles in action potential repolarization and the restitution of action potential duration (APD). The Luo-Rudy model of the ventricular action potential was used in the simulations. The single delayed rectifier K⁺ current in the model was replaced by I_{Kr} and I_{Ks} . Our results show that I_{Ks} is the major outward current during the plateau repolarization. A specific block of either I_{Kr} or I_{Ks} can effectively prolong APD to the same degree. Therefore, either channel provides a target for Class III antiarrhythmic drugs. In guinea pig ventricular cells, complete block of I_{Kr} does not result in early afterdepolarizations but I_{Ks} does. While, the underlying mechanism is not determined by the kinetics of I_{Kr} and I_{Ks} but their whole cell conductance. The computed APD restitution curve is consistent with the experimental behavior, displaying fast APD variation at short diastolic intervals (DI) and downward shift at longer DI with decrease of basic cycle length (BCL). Studying the ionic currents and their kinetic processes, we found that activation of both I_{Kr} and I_{Ks} is the primary determinant of the APD restitution at shorter DI, with I_{Ks} playing a minor role. The BCL-dependent shift of restitution at longer DI is primarily attributed to a change in intracellular Ca²⁺ concentration, which, in turn, causes different degree of calcium dependent inactivation of L-type calcium current and affects APD.

W-PM-B9

K⁺ CHANNELS IN PULMONARY ARTERIAL MYOCYTES ISOLATED FROM THE RAT. ((J.L. Turner, S. Albarwani*, and R. Z. Kozlowski)) University Department of Pharmacology, Mansfield Road, Oxford, OX1 3QT and *College of Medicine, Sultan Qaboos University, Al-Khod, Sultanate of Oman (spon. by A. Brading).

Using the patch-clamp technique we have compared K⁺ channel distribution in smooth muscle cells isolated from rat main pulmonary artery (MPA) and small pulmonary arteries (SPA; ~200-400 μ m diameter). Intracellular release of Ca²⁺ by flash-photolysis of nitr-5 reversibly activated an outward current (I_{out}) in cells isolated from MPA and SPA. The current density of I_{out} at a test potential of +30 mV was 190 ± 82 pA/pF ($n=4$; mean \pm s.e.m. throughout) and 111 ± 37 pA/pF ($n=5$) in cells isolated from the SPA and MPA, respectively. In inside-out patches from MPA the activity of a Ca²⁺ and ATP activated ($K_{Ca,ATP}$) channels predominated, openings of other channel types were rarely observed. In cells isolated from SPA two K⁺ channel types predominated: a $K_{Ca,ATP}$ channel and a K⁺ channel insensitive to voltage between -70 mV and +70 mV with a mean P_{open} of 0.9 ± 0.07 ($n=6$). This channel (K_{ins}) had a conductance of 185 ± 10 pS ($n=5$) in symmetrical 140 mM KCl and 110 ± 15 pS ($n=5$) under a quasiphysiological cation gradient. The P_{open} of K_{ins} channels (0.91 to 0.93 ; $n=5$) was not affected by $[Ca^{2+}]_i$ (~10 nM to 10 μ M) or by application of 500 μ M ATP to the intracellular membrane surface ($n=3$). These results show that K⁺ channels do not have a homogeneous distribution in the pulmonary vasculature.

W-PM-B6

THE LARGE CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM (K_{Ca}) CHANNEL REGULATES THE ACTION POTENTIAL IN SMOOTH MUSCLE FROM THE URINARY BLADDER ((T.J. Heppner and M.T. Nelson)) Univ. Vermont, Dept. Pharmacol., Colchester, VT 05401 (Spon. by Blair Robertson).

We tested the hypothesis that K_{Ca} channels are involved in the repolarization of the action potential of smooth muscle cells in guinea pig urinary bladder. To do this, we first identified and characterized single K_{Ca} channels in urinary bladder smooth muscle cells using the patch clamp technique. K_{Ca} channels were identified (internal K⁺; 140 mM, external K⁺; 6 mM) by their single channel conductance, activation by intracellular calcium, voltage-dependence and block by tetraethylammonium (TEA⁺). The amplitude of the unitary current was 6 pA at 0 mV and had a slope conductance of 122 pS. The open-state probability (P_o) of K_{Ca} channels increased as much as e-fold per 18 mV. Elevating intracellular calcium from 100 nM to 500 nM shifted the select midpoint of the relationship between P_o and membrane potential from +112 to +76 mV, respectively. TEA⁺ (200 μ M) induced a characteristic flickery block and reduced the mean channel amplitude by 67 %. Iberitoxin, a selective blocker of K_{Ca} channels in other preparations, reduced the open state probability of K_{Ca} channels in outside-out patches (0 mV) by 96%, which is consistent with a K_d of 4 nM. To examine the role of K_{Ca} channels in urinary bladder, the effects of iberitoxin on the action potential of smooth muscle cells were examined using intracellular microelectrodes. Iberitoxin (100 nM) significantly increased: 1) the spike amplitude from 47 mV to 61 mV, 2) duration of the spike at half-maximum from 12 ms to 38 ms, 3) 10%-90% decay of the spike from 7 ms to 36 ms, and 4) the duration and amplitude of the spike afterhyperpolarization by 60 % and 30 %, respectively. These results suggest that K_{Ca} channels have a unique role in smooth muscle from the urinary bladder and that the K_{Ca} channel may be a novel site for the modulation of bladder excitability. (Supported by Zeneca Pharmaceuticals Group and NIH)

W-PM-B8

COMPARATIVE EFFECT OF HYPOXIA ON CANINE PULMONARY AND RENAL ARTERIES. ((Craig H. Gelband, Sarena Keane, Helen Toland, and Joseph R. Hume)) Dept. of Physiol., Univ. of Nev., Reno NV 89557. (Spon. A. L. Bassett)

Hypoxia generally causes vasodilation in systemic vessels while constricting the pulmonary vasculature. Recently it was shown that hypoxia releases intracellular Ca²⁺ (Salvatera and Goldman, *AJP* 1993;264:L323) which may cause membrane depolarization by inhibiting K⁺ channels (Gelband *et al.*, *Circ. Res.* 1993;73:24). Yet the mechanism for the hypoxic vasodilation of systemic vessels has yet to be determined. The effect of hypoxia on isometric tension was investigated in rings of canine pulmonary and renal arteries. When the pulmonary artery (\pm endothelium) was precontracted with phenylephrine (Phe, 0.5 μ M) or KCl (80 mM), hypoxic challenge potentiated the Phe or KCl-induced contraction ($n=5$). Subsequent incubation with ryanodine abolished the hypoxic-induced contraction ($n=5$). Similar results were obtained with incubation of caffeine (10 mM) ($n=5$). Conversely when Phe (0.5 μ M)-induced contractions of the renal artery (+ endothelium) were challenged by hypoxia, an initial contraction occurred followed quickly by relaxation of the artery close to baseline ($n=6$). In renal artery with no endothelium present, a unique result occurred. After Phe (0.5 μ M) precontraction, hypoxic challenge resulted in partial relaxation (no initial contraction) followed by rhythmic oscillations in force. Application of caffeine (10 mM) or ryanodine (10 μ M) abolished these oscillations and relaxed the tissue completely ($n=6$). These results suggest that in both canine pulmonary and renal artery, the effect of hypoxia involves the sarcoplasmic reticulum. In the pulmonary vasculature this may be to initiate hypoxic pulmonary vasoconstriction (via Ca²⁺ release), while in the renal vasculature it may be to regulate intracellular Ca²⁺ cycling. (Supported by NIH HL-49254).

W-PM-C1

ACTIVATION OF CYCLIC NUCLEOTIDE-GATED ION CHANNELS. ((W. Altenhofen, J. Ludwig, D. Henn, E. Eismann, W. Weber, W. Bönigk, U.B. Kaupp)) Institut f. Biologische Informationsverarbeitung, Forschungszentrum Jülich, D-52425 Jülich, Germany. (Spon. by K.-W. Koch)

We have constructed chimeric cyclic nucleotide-gated (CNG) ion channels from bovine olfactory and rod photoreceptor CNG channels. The main determinants of the ligand efficacy reside in the N-terminus and in a linker between the pore and the nucleotide-binding site. The voltage dependence of activation is monitored as a sensitive measure of CNG channel function. It can be modified by mutations in the S4 segment. Deletion mutants show, that the domain C-terminal of the binding site is not required for channel gating. Based on statistical sequence analysis we identify a site in the binding domain that may be in contact with the transmembrane portion of the channel protein. A molecular channel model suggests a novel role for the S4 segment and predicts basic steps of CNG channel gating.

W-PM-C3

MOLECULAR DETERMINANTS OF AGONIST AND ANTAGONIST ACTION ON CYCLIC NUCLEOTIDE-GATED CHANNELS ((R. H. Kramer[†] & G. R. Tibbs[†])) [†]Dept. of Molec. & Cell. Pharmacol., University of Miami, Miami, FL 33101, and [‡]Ctr. for Neuro. & Behav., Columbia University, NY, NY 10032.

Photoreceptor and olfactory cyclic nucleotide-gated (CNG) channels are activated by cAMP and/or cGMP in a multi-step process involving both ligand binding and channel opening. We have identified cyclic nucleotide analogs that are competitive antagonists of channel activation by cAMP or cGMP. Thus the phosphorothioate derivative Rp-cAMPS binds to bovine photoreceptor CNG channels (RET channels) but does not lead to channel opening, while Rp-cAMPS is a partial agonist of catfish olfactory CNG channels (OLF channels). Here we report that Rp-cGMPs, while fully activating RET channels, is an antagonist of OLF channels. 8-p-chlorophenylthio Rp-cGMPs (8-pCPT-Rp-cGMPs) has similar effects, but is membrane-permeant and has a higher apparent affinity. To ascertain which part of the CNG channel protein determines whether a ligand is an agonist or an antagonist, we examined the effects of 8-pCPT-cGMPs on chimeric combinations between RET and OLF. We focussed on the carboxyl terminal domain, which contains a consensus cyclic nucleotide-binding site. RET channels containing the OLF C-terminal domain were antagonized by 8-pCPT-cGMPs, while OLF channels containing the RET C-terminal were fully activated by the analog. Thus the C-terminal domains of both RET and OLF can bind 8-pCPT-cGMPs, but only the RET domain couples binding to channel opening. We are currently examining specific regions of the C-terminal domain crucial for determining channel activation by this analog. Supported by NIH grant NS30695.

W-PM-C5

NICKEL ALTERS GATING OF CYCLIC NUCLEOTIDE-ACTIVATED CHANNELS ((Sharon E. Gordon and William N. Zagotta)) Dept. of Physiology and Biophysics and Howard Hughes Medical Institute, Univ. of Washington, Seattle, WA 98195.

We have examined the effects of Ni²⁺ on cyclic nucleotide-activated channels from rat olfactory epithelium and bovine rod photoreceptors expressed in *Xenopus* oocytes. As with the native rod channel, Ni²⁺ potentiated cGMP-activated currents in the expressed rod channel. With application of 10 μ M Ni²⁺, the apparent affinity for cGMP increased from 137 to 15 μ M, with only a small increase in the maximal current. With cAMP we observed an increase in apparent affinity and a ~50-fold increase in the maximal current. Ni²⁺ did not potentiate activation of the olfactory channel; rather, it decreased the maximal current and decreased the apparent affinity for both cGMP and cAMP. We localized the potentiation by Ni²⁺ in the rod channel to a single histidine residue following the S6 segment. The blocking action of Ni²⁺ on the olfactory channel localized to a nearby histidine found only in the olfactory channel. We propose a mechanism in which Ni²⁺ affects gating by stabilizing either the open (rod) or closed (olfactory) configuration of the channels.

W-PM-C2

CYCLIC GMP BINDING SITES WITHIN TWO DIFFERENT SUBUNITS OF THE RETINAL ROD cGMP-GATED CHANNEL

((Jeffrey W. Karpen, Robert Gramling, Robert J. Bert, and R. Lane Brown)) Dept. of Physiology, University of Colorado School of Medicine, Denver, CO 80262.

Ion channels from retinal rods and a variety of other cells are directly gated by cyclic nucleotides. Using a photoaffinity analog of cGMP, 8-p-azidophenacylthio-[³²P]cGMP, we have identified amino-acid residues of the 63-kDa channel subunit in close proximity to the guanine ring system. The labeled residues (val-524, val-525, and ala-526) would appear to form a hydrophobic cluster that lines the binding pocket. A model of the cGMP-binding site proposed by Kumar and Weber predicted that the labeled residues would be located on a segment of beta strand adjacent to the C₈ position of the guanine ring system. The model is therefore consistent with our photoaffinity-labeling result. Our results also provide biochemical evidence that a 240-kDa protein that co-purifies with the 63-kDa protein binds cGMP and is a second channel subunit. Sequencing of a specifically-labeled 8-kDa cyanogen-bromide fragment revealed a stretch of 16 amino acids that is identical to part of a protein recently discovered by Chen et al. and proposed to be a second subunit based on molecular genetic evidence. Furthermore, alignment of the amino acid sequences of the two cloned subunit proteins based on homology indicates that the specifically-labeled peptide from the 240-kDa protein is the corresponding fragment to that labeled from the original subunit. One goal for the future will be to determine how the binding of cGMP to each subunit type contributes to channel activation.

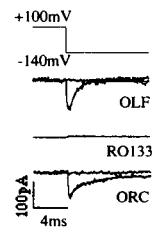
Supported by NIH grants EY09275 and EY06425.

W-PM-C4

SPONTANEOUS OPENING OF CYCLIC NUCLEOTIDE-GATED CHANNELS SUPPORTS AN ALLOSTERIC MODEL OF ACTIVATION

((G.R. Tibbs, E.H. Goulding & S.A. Siegelbaum)) Center for Neurobiology & Behavior, Columbia University, NY, NY 10032

Chimeric constructs of bovine photoreceptor (RET) and catfish olfactory (OLF) cyclic nucleotide-gated (CNG) channels suggest that a domain extending from the middle of the amino terminus through the S2-S3 loop (N-S2) determines the free energy change of channel activation (Goulding et al., submitted), with the RET N-S2 domain rendering channels harder to open than OLF. According to an allosteric model, the N-S2 domain should determine the spontaneous (ligand independent) open probability (s.o.p.) with OLF exhibiting a higher s.o.p. than RET. We relied on the voltage-dependent open CNG channel block by the Shaker ball peptide (Kramer et al., *Neuron* 12, 655-662) to trap spontaneously open channels in the open blocked state at +100 mV. Upon relief of block by hyperpolarization to -140 mV, patches containing OLF channels displayed a large tail current, indicating a s.o.p. of 0.2-1x10⁻⁶. No tail current was observed in patches containing RET or a chimeric RET channel, RO133, that contains the OLF H5 region (and hence the same affinity as OLF for peptide). However, a chimeric channel (ORC) with the OLF amino-transmembrane domains (and hence N-S2 domain) and RET carboxy binding domain did display a large tail current. These results indicate that: 1. Unliganded CNG channels can open, 2. OLF opens more readily than RET, and 3. S.o.p. may be determined by the N-S2 domain.



W-PM-C6

C-TERMINUS INVOLVEMENT IN THE GATING OF CYCLIC NUCLEOTIDE-ACTIVATED CHANNELS AS REVEALED BY Ni²⁺ AND NEM. ((J.T. Finn, J. Li and K.-W. Yau)) Howard Hughes Med. Inst. and Dept. of Neurosci., Johns Hopkins School of Medicine, Baltimore, MD 21205.

Previous work has shown that the rod cGMP-gated channel can be potentiated by Ni²⁺ [Karpen et al., *J. Gen. Physiol.* 101:1-25, 1993] and N-ethylmaleimide (NEM) [Donner et al., *Exp. Eye Res.* 51:97-105, 1990; Balakrishnan et al., *Biophys. J.*, 57:371a, 1990]. We have examined the molecular mechanisms underlying these potentiations of the rod channel, and have carried out similar experiments on the cAMP/cGMP-gated channel mediating olfactory transduction. cDNAs encoding subunit 1 (or α) of the channels were expressed in HEK293 cells and the resulting channel activity was studied in excised, inside-out patches. Incubation with either 3 μ M Ni²⁺ or 2 mM NEM shifted the apparent affinity ($K_{1/2}$) of the rod channel for cGMP from approximately 60 μ M to 10 μ M. The effects of Ni²⁺ and NEM were not additive when the two were applied simultaneously. Ni²⁺ had no effect on the olfactory channel, while NEM shifted the $K_{1/2}$ from 3 μ M to 0.8 μ M. Experiments on chimeric and point-mutant channels showed that the cytoplasmic C-terminus was involved in the effects of both Ni²⁺ and NEM. In the rod channel, the mutation H418A abolished potentiation by Ni²⁺ while the mutation C479A abolished potentiation by NEM. The corresponding mutation C460A in the olfactory channel likewise abolished NEM potentiation. In experiments on the rod channel with cAMP, treatment with Ni²⁺ or NEM increased the maximum current elicited by cAMP almost 7-fold, although the single channel conductance was not changed. Thus, it appears that the binding of Ni²⁺ or NEM to the C-terminus of the rod channel potentiates the channel by increasing its gating efficacy. (Our results on Ni²⁺ potentiation agree with those recently obtained in the laboratory of W.N. Zagotta.)

W-PM-C7

GABA_A-RECEPTOR FUNCTION IS NOT AFFECTED BY CALCIUM INFLUX IN RAT HIPPOCAMPAL CA1 CELLS. ((M. Džoljic, W. Wadman and B. Van Duijn)) Academic Medical Center, University of Amsterdam, Dept. of Anaesthesiology, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands and Dept. of Experimental Zoology, University of Amsterdam, The Netherlands.

Reported changes in GABA-receptor function as a result of intracellular Ca^{2+} concentration increase, may have profound effects on our look at neuronal excitability. In this study the effects of Ca^{2+} influx on activity of GABA_A receptors were evaluated. Under whole-cell voltage clamp conditions increasing depolarisations induced increasing Ca^{2+} influxes. After each depolarisation, the GABA_A agonist muscimol was applied. Cl^{-} currents seemed reduced in proportion to Ca^{2+} influx. However, replacing Ca^{2+} by Ba^{2+} showed the same results as did inverting the voltage clamp protocol. Our data show that not Ca^{2+} influx but the time lap between the different muscimol application is determining the magnitude of the Cl^{-} conductance. Currently we are investigating the effects of volatile anaesthetics, e.g. nitrous oxide, on the GABA_A-receptor function.

W-PM-C9

ACTIVATION OF 5-HT₂ RECEPTORS EXPRESSED IN XENOPUS OOCYTES DOES NOT INCREASE CYTOPLASMIC Ca^{2+} LEVELS. ((P. Gilon and J. L. Yakel)) Lab. of Cellular and Molecular Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709. USA (Spon. by M. Sumner)

The issue of Ca^{2+} permeability of the 5-HT₂ receptor channel is still debated. Therefore, the wildtype 5-HT₂ receptor was expressed in *Xenopus* oocytes, and two-electrode voltage-clamp and fura-2 techniques were used to simultaneously monitor the current induced by the activation of the 5-HT₂ receptor and the cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). The application of serotonin (5-HT; 50 μM) in a 'physiologic' bathing solution containing Ca^{2+} (1.8 mM) and Na^{+} (96 mM) elicited inward currents of more than 1 μA at a potential of -90 mV. In 16 oocytes in which the 5-HT response was > 100 nA, there was no detectable change in $[\text{Ca}^{2+}]_i$ in 14 of these oocytes; in the other two, the increase in $[\text{Ca}^{2+}]_i$ was < 3 nM. As a control to test for changes in $[\text{Ca}^{2+}]_i$ levels, the injection in a nominally Ca^{2+} -free medium of an analog of inositol 1,4,5-trisphosphate ((1,4,5)IP₃), (2,4,5)IP₃, produced a large increase in $[\text{Ca}^{2+}]_i$ levels due to the liberation of Ca^{2+} from intracellular stores. This response was followed by a sustained elevation of $[\text{Ca}^{2+}]_i$ upon restoring extracellular Ca^{2+} (i.e. capacitative Ca^{2+} entry). It is concluded that for wildtype 5-HT₂ receptors expressed in *Xenopus* oocytes and under the 'physiologic' ionic conditions used here, the permeability of these receptor channels for Ca^{2+} is either extremely low or non-existent.

RECEPTORS AND KINASES

W-PM-D1

POSSIBLE INTERACTIONS BETWEEN TCR, MHC CLASS I OR II MOLECULE, AND THE PROCESSED PEPTIDE. ((G. Johnson and T. T. Wu)) EMBECB and BME, Northwestern University, Evanston, IL 60208.

Using variability plots, we have analyzed possible interactions between T-cell receptor for antigen (TCR) alpha and beta chains, major histocompatibility complex (MHC) class I A1 and A2 regions or class II A1 and B1 regions, and the processed peptide. Variability plots of the third complementarity determining region (CDR) of TCR alpha and beta chains show distinct peaks, similar to those of immunoglobulins, suggesting that they interact with the processed peptide exclusively. On the other hand, the variability plot of CDR1 of TCR beta chains shows a "split" peak, suggesting that the middle portion of this CDR1 interacts with a relatively conserved portion of the MHC molecules. If the TCR chains have the standard immunoglobulin fold, CDR1 would be able to interact with the C-terminal end of the helical regions of MHC molecules. Variability plots of MHC class I A1 and A2 regions and MHC class II A1 and B1 regions indicate that MHC class I A2 and MHC class II A1 have lower variability in that end of the helices. Therefore, we propose that TCR beta chain binds the processed peptide and MHC class I A2 region or MHC class II A1 region, and TCR alpha chain binds the processed peptide and MHC class I A1 region or MHC class II B1 region. CDR1, the "fourth" loop and CDR2 of both chains form a continuous surface, interacting with the side of the processed peptide and touching the MHC helix.

W-PM-C8

MAPPING THE PICROTOXIN BINDING SITE IN THE GABA-A RECEPTOR CHANNEL USING CYSTEINE MUTAGENESIS. ((Myles H. Akabas and Ming Xu)) Center for Molecular Recognition, Columbia University, New York, NY 10032.

We have identified the residues from the M2 membrane spanning segment of the rat GABA_A receptor $\alpha 1$ subunit which line the ion channel by mutating them, one at a time, to cysteine. We expressed the mutant $\alpha 1$ subunit with wild type $\beta 1$ & $\gamma 2$ subunits in *Xenopus* oocytes. We probed the susceptibility of the engineered cysteine to covalent modification by small, charged, sulfhydryl-specific reagents added extracellularly. We assume that only residues lining the channel would be susceptible to modification and that such modification would irreversibly alter conduction. We showed that I271, T268, T265, L264, T261 and V257 are exposed in the channel. Picrotoxin is thought to block conduction by binding in the channel. When picrotoxin is coapplied with the sulfhydryl-specific reagents in the presence of GABA it protects V257C from modification. Although picrotoxin blocks conduction in the T261C mutant, it does not protect T261C from modification. Reaction of methanethiosulfonate-ethylammonium with picrotoxin blocked T261C channels relieves the block. In an α helix, T261 would be located 6 Å closer to the extracellular end of the channel than V257. These results demonstrate that picrotoxin binds in the channel lumen at the level of V257. Supported by NIH NS30808, AHA Grant-in-Aid and Klingenstein Award in Neuroscience.

W-PM-D2

SYNERGISTIC EFFECT OF MUSCARINIC AND P₁-PURINERGIC RECEPTOR ACTIVATION ON $[\text{Ca}^{2+}]_i$ IN INTACT RABBIT CILIARY BODY EPITHELIUM ((N.A. Farahbakhsh and G.L. Fain)) Dept. Physiol. Science and Jules Stein Eye Institute, UCLA, Los Angeles, CA 90024-1527.

We have recently reported that both acetylcholine (ACh) and epinephrine (Epi) can by themselves induce modest increases in $[\text{Ca}^{2+}]_i$ in the epithelial cells of the ciliary body, but a massive increase in $[\text{Ca}^{2+}]_i$ can be observed when both are applied together. This synergistic effect is mediated by muscarinic cholinergic receptors (for ACh) and α_2 -adrenergic receptors (for Epi) (*J. Physiol.* 477: 215-221, 1994). We now show that the purinergic agonist adenosine (Ado, 0.1-1 μM) also has synergistic effects on $[\text{Ca}^{2+}]_i$, when applied simultaneously with ACh (10 μM). This latter effect is caused by the activation of A₁-type adenosine receptors: it can be blocked by the specific A₁ antagonist DPCPX (0.1-1 μM), but not by the A₂-specific antagonist DMPX (0.1-1 μM). A large increase in $[\text{Ca}^{2+}]_i$ can be induced by 10 μM ACh in combination with 0.1 μM of the specific A₁ agonists CHA, CPA and R(-)-PIA, but not together with 0.1-1 μM of the A₂-specific agonists CV-1808 or CGS-21680. A₂ agonists also failed to raise $[\text{Ca}^{2+}]_i$, when applied alone. Finally, the synergistic increase in $[\text{Ca}^{2+}]_i$ is mediated by a pertussis toxin (PTX)-sensitive G-protein linked to A₁ adenosine (and α_2 -adrenergic) receptors: PTX-treated cells showed an increase in $[\text{Ca}^{2+}]_i$ in response to simultaneous application of ACh and Ado (or Epi) no larger than that induced by ACh alone. Supported by NEI grants EY06969 and EY07058.

W-PM-D3

MOLECULAR DYNAMICS SIMULATIONS TO MODEL THE PROPERTIES OF CONSTITUTIVELY ACTIVE GPCR MUTANTS. ((K. Haydock and H. Weinstein)) Department of Physiology and Biophysics, Box1218, Mount Sinai School of Medicine, New York, NY 10029.

To elucidate the signal transduction mechanism whereby ligand binding induces a receptor to couple to a G-protein, we are studying structural elements of mutant $\alpha 1B$ -adrenergic receptors which can couple to G-proteins even in the absence of ligand. In this receptor the replacement of a single alanine at position 6.34(293) by any other amino acid has been shown to produce constitutive activation (Kjelsberg et al., 1992, J. Biol. Chem. 267, 1430). This effect may occur because the mutation stabilizes a conformational deformation of the receptor that is similar to the deformation that occurs upon ligand binding. The mutation is located in a putative intracellular helical extension to helix 6, which is quite distant from the supposed ligand binding site. We hypothesize that the simplest mechanism for constitutive activation is that the mutation induces a conformational change in this helix. Alternatively, or in addition, the mutation may induce a more general conformational change involving other helices or loop regions. To test the simplest case, we have made a molecular dynamics simulation of an isolated, 31 residue long helix 6 in a membrane model. The membrane is modelled as a 40 Å thick layer of carbon tetrachloride molecules representing the hydrophobic interior, capped by water layers representing the polar regions, similar to the model of Guba and Kessler (1994, J. Phys. Chem. 98, 23). The simulations are carried out in a 30x30x66 Å box with periodic boundary conditions. By repeating the same simulation on helices with various mutant and wild-type sequences, we have been able to identify conformational effects induced by mutation. Supported by an Aaron Diamond Fellowship (KH) and NIH grant DA00060.

W-PM-D5

MITOGEN ACTIVATED PROTEIN KINASES IN MAMMALIAN SMOOTH MUSCLE. ((I. Yamboliev, J. Pliml and W. T. Gerthoffer)) Dept. Pharmacology, Univ. Nevada School of Medicine, Reno, Nevada, 89557-0046.

Mitogen activated protein kinase (MAPK) activities were compared in resting and stimulated canine tracheal smooth muscle using three methods. Myelin basic protein (MBP) was employed as the substrate in a gel reconstitution method according to Geahlen *et al.* (Anal. Biochem. 153: 151-158, 1986). Kinase activities in SDS extracts were separated by SDS-PAGE (12%) and visualized by autoradiography. MBP kinase activities at 38 and 44 kDa increased 2.03 ± 0.52 (SEM) and 2.08 ± 0.47 fold respectively after 5 min stimulation with 1 μ M carbachol. MAPK activity was also assayed in crude homogenates using the synthetic nanopetide APRTPGGRR as substrate. The kinase reaction was stopped by spotting a sample onto P81 phosphocellulose paper (Whatman), washing with 75 mM H_2PO_4 and counting. Total MAPK activity increased 2.00 ± 0.73 fold in stimulated muscles. MAPK isoforms were separated by anion exchange HPLC using a Mono-Q HR 5/5 column and a linear NaCl gradient (0 to 0.4M, 0.5 ml/min). MAP kinase activities assayed using the MAPK substrate peptide eluted as two distinct peaks in the range 0.15-0.30M NaCl. The peaks were identified on Western blots as 38 and 44 kDa MAP kinases. Kinase activities of both peaks increased after stimulation with carbachol. Therefore, three different methods report similar activation of MAP kinases during contraction of smooth muscle. Supported by NIH grant HL48183.

W-PM-D7

ENDOTHELIN-1 INHIBITS L-TYPE Ca^{2+} -CURRENTS ENHANCED BY ISOPROTERENOL IN GUINEA PIG VENTRICULAR MYOCYTES. ((L.-H. Xie, M. Horie, A. F. James* and S. Sasayama)) Kyoto University, Kyoto 606-01, and *NIPS, Okazaki 444, Japan. (Spon. by M. Nishimura)

Endothelin-1 (ET-1) is a peptide hormone released from vascular and endocardial endothelial cells, especially under ischemic conditions. Although ET-1 has been shown to exert a positive inotropic effect when administered alone, it also inhibits the positive inotropic action of catecholamines. Since L-type Ca^{2+} -channel currents (I_{CaL}) play a key role in modulating cardiac inotropy, we studied the effects of ET-1 on I_{CaL} in guinea pig ventricular cells. Whole-cell currents were recorded at $-36^\circ C$ in myocytes voltage-clamped and internally dialyzed with Cs-rich, K-free pipette solution (100 μ M GTP; 10 mM BAPTA). Fast Na- and T-type Ca^{2+} -channel currents were inactivated by applying a brief prepulse to -40 mV from -80 mV holding potential. I_{CaL} were measured as the difference between peak inward current and steady state at the end of 300 ms test pulse to 0 mV. ET-1 (20 nM) suppressed the basal I_{CaL} to $79 \pm 8\%$ of control. Bath application of isoproterenol (ISO; 10 nM) enhanced I_{CaL} to $192 \pm 28\%$. The ET-1 concentration-dependently inhibited this ISO-stimulated I_{CaL} with an IC_{50} of 168 pM. The inhibitory actions of ET-1 were antagonized by BQ123 (300 nM), a specific ET_A receptor antagonist. Histamine-enhanced I_{CaL} was also suppressed by ET-1, but I_{CaL} enhanced by internal cAMP (1 mM) was unaffected by ET-1. Pre-incubation of myocytes with pertussis toxin (PTX, >60 min at 3 μ g/ml) completely abolished the action of ET-1. Thus, under our recording condition, stimulation of ET_A receptors by ET-1 inhibits I_{CaL} via PTX-sensitive G-proteins. This action of ET-1 is consistent with an inhibition of the positive inotropic effect of catecholamines. (Supported by Grants-in-Aid for Scientific Research from the Japan Ministry of Education, Science and Culture)

W-PM-D4

INHIBITION OF A CARDIAC SARCOPLASMIC RETICULUM ASSOCIATED PROTEIN KINASE A-LIKE KINASE BY CALCIUM IONS ((Colyer, John)) Dept. of Biochemistry & Molecular Biology, University of Leeds, Leeds LS2 9JT, UK (Spon. by J. Chad)

A number of protein kinases thought to be involved in calcium homeostasis are associated with the cardiac muscle sarcoplasmic reticulum (CSR). One of these enzymes, here called the CSR-kinase, displays properties like those of the catalytic subunit of the cAMP-dependent kinase in that it is independent of cyclic nucleotide concentration, utilises similar protein substrates (including phospholamban), and is inhibited by the specific PKA peptide inhibitor, PKI(5-22amide). It differs from the A-kinase in one respect, namely that the SR-kinase is inhibited by Ca^{2+} , with an IC_{50} of $\approx 30 \mu$ M. The effective range of $[Ca^{2+}]$ reflects that believed to occur in the lumen of the CSR, raising the possibility that the CSR-kinase monitors the Ca^{2+} -status within the CSR and is activated upon depletion of this store. To test this hypothesis, cardiac myocytes were treated with three interventions designed to deplete the CSR of calcium. Thapsigargin (10 μ M) promoted phosphorylation of phospholamban whereas ryanodine (10 μ M) and EGTA (5mM) did not. Consistent with these latter results, loading CSR vesicles with Ca^{2+} to 1-2mM *in vitro* also failed to inhibit the SR-kinase as determined by a peptide phosphorylation assay. In conclusion, cardiac SR contains a novel kinase related to the cAMP-dependent protein kinase, which is regulated by Ca^{2+} , but not from a luminal source.

W-PM-D6

PLASMIN-PLATELET INTERACTION INVOLVES ACTIVATION OF THROMBIN RECEPTORS (M. Kimura, T.T. Andersen, J.W. Fenton, II, W.F. Bahou and A. Aviv) Hypertension Research Center, Univ. of Med. & Dentistry of NJ, Newark, NJ, 07103 (Spon. by J. Reeves)

Plasmin (a serine protease) exerts a dual effect on platelet function. At high concentrations plasmin raises the cytosolic Ca^{2+} and activates platelets. However, it also attenuates the thrombin-mediated rise in Ca^{2+} and platelet activation. It was proposed that this duality reflects the coupling of the plasmin receptor to downstream signaling systems, including phospholipase C, protein kinase C, and tyrosine kinase, which render platelet less sensitive to further action of thrombin. We tested the concept that the dual effect of plasmin is mediated, at least partially, via a direct action on thrombin receptors. This action generates the tethered peptide, the terminal 6 amino acid sequence of which is SFLLRN. Platelets treated with medium containing the synthetic peptide (SP) γ -FPRSFLLRNPNKYEPP demonstrated no alteration in the Ca^{2+} signal. However, prior treatment of the medium with either plasmin or thrombin resulted in a pronounced rise in the Ca^{2+} upon exposure of platelets to this medium. The increase in the Ca^{2+} was related in a time- and dose-dependent fashion to the preincubation of the SP with either agonist. The sequential addition of plasmin after SP to platelet suspension resulted in a robust rise in Ca^{2+} , which was dose-dependent (with respect to both plasmin and the SP). Amino acid analysis of platelet supernatant after treatment with thrombin or plasmin revealed that both agonists cleaved similar cellular targets. Furthermore, platelets treated with either thrombin or plasmin demonstrated diminished binding of the anti-thrombin receptor antibody (anti-TR 160), suggesting cleavage or internalization of the thrombin receptor by either agent. Our data therefore suggest that plasmin exerts its effect on platelets by enzymatic action on thrombin receptors. This results in partial platelet activation and the attenuation of their response to thrombin.

W-PM-D8 (See Th-Pos469)

W-PM-E1

MUTATIONS IN THE TRANSMEMBRANE DOMAIN OF Na⁺,K⁺ ATPase α SUBUNIT ALTERS AFFINITY FOR EXTERNAL K⁺. ((S. Yamamoto, *T. Kuntzweiler, *J. Lingrel, *E. T. Wallick and *A. Yatani))

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The ATP-driven Na⁺,K⁺ pump extrudes 3 Na⁺ in exchange for 2 K⁺ and therefore generates an outward Na⁺ pump current (I_p). It has been proposed that highly conserved negatively charged residues (E327 and D925) located in the transmembrane domains, H4 and H7 of the Na⁺,K⁺ ATPase (NKA) α subunit involved in cation binding. To study the functional role of these residues: neutral amino acids were singly substituted in a ouabain-resistant isoform (rat $\alpha 2^*$), stable HeLa cell lines expressing either wild-type or the mutant cDNAs were established and I_p was measured by whole-cell patch-clamp technique which allowed rapid external solution changes. The endogenous ouabain-sensitive HeLa NKA was selectively inhibited by growing the cells in the presence of 1 μ M ouabain. Both wild-type and mutant cells displayed marked external K⁺-dependent I_p activation; however, the apparent affinity (K_d) for K⁺ was modified by the mutations. The rank order of K⁺ affinity was D925N > Wild-type >> D925L \approx E327Q. The apparent decrease in K⁺ affinity was due both to a slower association and a faster dissociation rate. These results suggest that the sites E327 and D925 participate in the interaction of the enzyme with external K⁺. (Supported by AHA93012860, HL28573, HL41496 and HL50613).

W-PM-E3

ASSAY OF Na,K-ATPASE MUTANTS EXPRESSED IN YEAST CELLS BY MEDIUM ¹⁸O EXCHANGE. ((L. D. Faller, V. N. Kasho and R. A. Farley)) USC School of Medicine and CURE, UCLA/VAMC Wadsworth, West Los Angeles, CA 90073.

Lack of endogenous Na,K-ATPase activity makes yeast cells an ideal expression system for sodium pump. Unfortunately, the usefulness of the yeast expression system is limited by the low signal to noise ratio of some of the existing functional assays resulting from low expression of sodium pumps (<0.1% of protein). We report signal to noise ratios > 10 for assay of sodium pump mutants expressed in yeast cells by medium ¹⁸O exchange. Other advantages of medium ¹⁸O exchange are that the mechanism of the reaction is understood, exchange is catalyzed by a partial reaction in the overall cycle, both the exchange rate and the isotopomer distribution can be measured, and the exchange parameters depend dramatically on pH. A model based on sequence homology for the ATP binding site of P-type pumps has been tested by changing amino acids in the putative phosphate and ribose binding domains. Mutants have been found with normal ATPase and ¹⁸O exchange activity; with decreased ATPase and ¹⁸O exchange activity; with reduced ATPase, but normal ¹⁸O exchange activity; and with normal ¹⁸O exchange rate, but different isotopomer distribution and enhanced ATPase activity. Supported by NIH, NSF, and VA Merit Review.

W-PM-E5

CHARGE TRANSLOCATION MEDIATED BY THE Na/K PUMP IN INTERNALLY-DIALYZED SQUID GIANT AXON. ((M. Holmgren, F. Bezanilla, D.C. Gadsby, P. De Weer and R. F. Rakowski)) Marine Biological Laboratory, Woods Hole, MA 02543.

It has been suggested that the main voltage dependent step(s) in the electroneutral Na/Na exchange mode of operation of the Na/K pump results from charge movement in an extracellular access channel. Giant axons from the squid *Loligo pealeii* were internally dialyzed and externally superfused with K- and Cl-free solutions similar to those used by Gadsby et al., 1993 (Science, 260:100) but without internal ADP. In the absence of ADP, the pump is distributed mainly among states in which the ion binding sites face the extracellular medium. Under these conditions, presteady-state currents were elicited by voltage steps from a holding potential of 0 mV to potentials over the range -170 to +70 mV. Current records in the presence of 10 μ M dihydrodigenin (H₂DTG) were subtracted from current records obtained in its absence. H₂DTG-sensitive transient currents at 18°C to 20°C consisted of a fast (< 10 μ s) and a slow (> 500 μ s) components. The slow component was dependent on extracellular Na concentration ([Na]_o), but the fast component was present in the absence of Na (TMA substitution) and even in the absence of monovalent ions (mannitol substitution). The amount of slow charge moved (Q_s) was a sigmoidal function of voltage with an apparent valence of 1.17 \pm 0.05 (\pm SEM, n = 8). The mid-point voltage of Q_s was a function of [Na]_o with values of 7.3 \pm 1.0, -27.2 \pm 0.8 and -43.1 \pm 0.6 mV for [Na]_o of 400, 200 and 100 mM, respectively. The relaxation rate of Q_s (k₃) increased steeply at negative potentials but declined to a minimum voltage independent rate of ~ 100 s⁻¹ at positive potentials. At negative (but not at positive potentials), the relaxation rates also depended on [Na]_o, and were faster at higher Na concentrations. The presence of 5 mM internal ADP reduced the total amount of charge moved by $\sim 50\%$ without any appreciable effect on k₃. These results are consistent with the hypothesis that the principal charge movement in Na/Na exchange accompanies the migration of Na ions between the binding site(s) in the Na/K pump and the external medium. Supported by Grass Foundation and NIH grants: GM 30376, HL 36783, NS 11223, NS 22979.

W-PM-E2

MUTATION OF GLU-327 AND ASP-925 OF Na⁺,K⁺-ATPase (NKA) REVEALS POSITIVE COOPERATIVITY IN Rb⁺ UPTAKE. ((E. T. Wallick, L. A. Millette, C. L. Johnson, J. B. Lingrel, and K. Tepperman)) University of Cincinnati, Cincinnati OH 45267-0575. (Spon. by J. Stout)

Mutation of Glu-327 and Asp-925 residues in NKA alters the affinity of NKA for potassium as assessed by measurement of ATPase activity (Jewell-Motz, E. & Lingrel, J.B. Biochemistry, 1993, 32,13523-13530). A competition assay was used to measure ⁸⁶Rb uptake into HeLa cells transfected with mutations of a rat $\alpha 2$ isoform (WT) modified to be ouabain insensitive. Experiments were carried out in the presence of 1mM furosemide (to block Na,K-Cl-cotransporter) and 1 μ M ouabain (to inhibit endogenous HeLa NKA). ⁸⁶Rb uptake into cells containing mutants E327Q and E327L were best fit by positively cooperative models (sequential or random) which bind two K⁺ ions. Compared to WT and mutant D925N, the mutants at position 327 have a lower affinity for K⁺ (cf Table). Lowering external Na⁺ from 140 mM to 15 mM increased the affinity for K⁺. Interestingly, models which assume that only doubly occupied K⁺ sites are able to carry out transport do not fit the data very well. This finding implies that enzyme with a single K⁺ bound is able to undergo the conformational change that leads to transport.

Cell Type	K ₁	K ₂	K ₁ /K ₂
WT	2.93 \pm 0.98	0.81 \pm 0.46	3.6
D925N	1.90 \pm 0.72	0.70 \pm 0.60	2.3
E327Q	15.1 \pm 7.7	0.87 \pm 0.38	17
E327L	15.3 \pm 2.1	3.28 \pm 0.59	4.7

(Supported by HL-50613 (E.T.W.) & HL-28573 (J.B.L.))

W-PM-E4

TRANSIENT AND STATIONARY Na/K-ATPase CURRENTS IN EXCISED PATCHES FROM RAT HEART CELLS INDUCED BY AN ATP CONCENTRATION JUMP.

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The giant patch technique (Hilgemann, 1989) made it possible to measure currents generated by ion pumps or exchangers in excised patches. Under appropriate conditions (which inhibit all other currents by channels or exchangers) an ATP dependent outward current can be demonstrated in inside-out excised patches from plasmamembrane of isolated rat heart myocytes.

We showed that this outward current exhibits all the properties expected for a Na/K-pump generated current like: complete, reversible inhibition by orthovanadate, dependencies on cytoplasmic Na and MgATP and on pH.

To generate a fast rise of ATP concentration, we photolyzed caged ATP with a 10 ns laser flash of 308 nm wavelength (Fendler et al, 1987). At 24°C and pH 7.4 the resulting current rose with a time constant of about 30 ms to a stationary level, as expected for the known pH dependent release of ATP from caged ATP. At pH 6.3 however, where ATP is released within about 1 ms, a transient outward current with a rise time of a few ms was observed, followed by a decay to a stationary pump current. This signal was Na and ATP dependent and could be abolished by ortho-vanadate. In contrast to the voltage jump induced transient pump currents observed under Na/Na exchange conditions (Nakao & Gadsby, 1986), no extracellular Na (i.e. in the pipette) was needed for this transient pump current. The results will be discussed on the basis of the Albers-Post reaction cycle of the Na/K pump.

(Supported by the Deutsche Forschungsgemeinschaft.)

W-PM-E6

A DIRECT ASSAY FOR ATP BINDING TO Na⁺,K⁺-ATPase (NKA) USING ³⁵S-ATPyS (AyS). ((C. L. Johnson, C. G. Johnson, and E. T. Wallick)) Dept. Pharmacology & Cell Biophysics, University Cincinnati, Cincinnati OH 45267-0575. (Spon. by T. Kirley)

Cardiac glycoside binding to NKA is affected by ATP, Na⁺, and K⁺. To examine ATP regulation, we developed an AyS binding assay on purified NKA from sheep kidney. In the presence of Na⁺ and Mg²⁺, AyS saturation assays indicated the presence of a high affinity component (4.4 \pm 1.0 nM) as well as a lower affinity component (142 \pm 19 nM, N = 4). The high affinity component represented a small fraction (about 5%) of total binding and was not stimulated by Na⁺. In contrast, at high AyS concentrations (> 100 nM), which represents primarily the low affinity component, Na⁺ markedly stimulated binding with an EC₅₀ of 4.8 \pm 0.3 mM and a Hill coefficient of 3.1 \pm 0.2 (N = 4), consistent with the binding of Na⁺ to three sites. In the absence of Na⁺, K⁺ inhibited AyS binding with an IC₅₀ of about 100 mM. However, in the presence of Na⁺, a second inhibition component was observed with an IC₅₀ of 0.16 \pm 0.02 mM and a Hill coefficient of 2.20 \pm 0.06 (N = 3), consistent with the binding of two K⁺ ions. Ouabain caused a marked stimulation of AyS binding and increased its affinity by about a factor of ten. These preliminary results suggest that AyS binding may be a useful tool for future studies of the cardiac glycoside-NKA interaction. (Supported by HL 50613)

W-PM-E7

TWO-DIMENSIONAL STRUCTURE OF THE *NEUROSPORA CRASSA* PLASMA MEMBRANE H^+ -ATPASE AS DETERMINED BY ELECTRON CRYO-MICROSCOPY. ((M. Cyrklaff¹, M. Auer¹, W. Kühlbrandt¹, and G. A. Scarborough²)) ¹European Molecular Biology Laboratory, Heidelberg, Germany, and ²University of North Carolina, Chapel Hill, NC, 27599.

Large, well-ordered two-dimensional crystals of the dodecylmaltoside complex of the *Neurospora crassa* plasma membrane H^+ -ATPase grow rapidly on the surface of a polyethylene glycol-containing mixture similar to that originally developed for growing three-dimensional crystals of this integral membrane transport protein. Negative stain electron microscopy of the crystals shows that many are single layers. Cryoelectron microscopy of unstained specimens indicates that the crystals have a hexagonal layer group (p6) with unit cell dimensions of $a=b=167$ Å. Image processing of selected electron micrographs has yielded a projection map at 10.3 Å resolution. The repeating unit of the ATPase crystals comprises six 100 kDa ATPase monomers arranged in a symmetrical ring. The individual monomers in projection are shaped like a boot. These results provide the first indications of the molecular structure of the H^+ -ATPase molecule. They also establish the feasibility of precipitant-induced surface growth as a rapid, simple, alternative to conventional methods for obtaining two-dimensional crystals of integral membrane proteins.

W-PM-E9

THE MITOCHONDRIAL ATP SYNTHASE OF *TRYPANOSOMA BRUCEI*: REGULATION AND GENETIC MAPPING ((S.V. Brown, T.B. Chi, M. Tanaka, and N. Williams)) SUNY at Buffalo, Buffalo, NY 14214

The mitochondrial ATP synthase of the parasitic protozoan, *Trypanosoma brucei* has been the focus of study in this laboratory. The F_1 component has a typical $\alpha_3\beta_3\gamma\delta\epsilon$ structure with polypeptide molecular weights of 55, 42, 32, 22, and 17 kD. The F_0 has been less well characterized but appears to be comprised of at least 4-5 subunits. An endogenous inhibitor peptide has recently been shown to be associated with the complex. Regulation of the ATP synthase occurs on a number of levels. Both the F_1 and F_0 components are present at the highest levels in the procyclic [insect] stage of the *Trypanosoma brucei* which is most active in oxidative phosphorylation and at the lowest level in the early bloodstream form which is highly glycolytic. In contrast, the inhibitor peptide is at highest levels in the early bloodstream form and in the lowest levels in the procyclic stage. This suggests a tight regulation of the ATP synthase both in terms of the amount of enzyme present and in the activity that the enzyme may express in the cell. We are currently trying to determine whether regulation occurs at the level of transcription for several of the ATP synthase components [α , β , and the inhibitor peptide]. We are also mapping the genes for these proteins to the *Trypanosoma brucei* chromosomes to determine whether they are physically linked.

Ca²⁺ CHANNEL MODULATION

W-Pos1

CYCLIC AMP-MEDIATED PHOSPHORYLATION OF THE L-TYPE CALCIUM CHANNEL BETA-SUBUNIT IN INTACT MYOCARDIUM: ((H. Haase, P. Karczewski and E.G. Krause)) Max Delbrück Center for Molecular Medicine, 13122 Berlin, Germany. (Spon. by I. Morano)

Interaction between the α_1 - and β -subunits determines basic properties of L-type Ca^{2+} channels. We studied whether the β -subunit undergoes cAMP-mediated phosphorylation in intact dog hearts and may thereby regulate channel activity. β -subunits were phosphorylated in native membranes, solubilized, and subsequently immunoprecipitated with a polyclonal antibody generated against the deduced carboxy terminal sequence of the cardiac β -subunit. In preparations from dog hearts which were depleted from endogenous catecholamines by reserpine treatment (0.5 mg/kg, 2 days), the cyclic AMP-dependent protein kinase induced substantial [³²P]-phosphate incorporation into the β -subunit (0.7 mol P_i per mol dihydropyridine receptor). Using the back-phosphorylation technique (1), we found that in intact myocardium the β -subunit was fully phosphorylated *in-vivo* in response to cAMP elevating agents (isoprenaline, 10 μ g/kg; noradrenaline, 10 μ g/kg). Since the *in-vivo* phosphorylation is inversely related to the *in-vitro* [³²P]-incorporation, a close correlation was found among the rise in cAMP and β -subunit phosphorylation as well as positive inotropic and chronotropic responses. We suggest that in canine myocardium phosphorylation of the Ca^{2+} channel β -subunit is involved in the cAMP-mediated control of channel opening.

(1) Mundina-Weilemann et al. (1991) J Biol Chem 266, 4067-4073.

W-PM-E8

MODELING THE MEMBRANE DOMAINS OF ION-TRANSPORTING ATPases. ((K.B. Munson and G. Sachs)) UCLA Department of Medicine, VMAC/ West L.A., L.A., CA. 90073.

We have built molecular models for the membrane domains of the catalytic polypeptides from several phosphorylating (P-type) ion-transporting ATPases. The purpose is to identify residues that may affect ion binding and inhibitor specificity. The models include only the first six transmembrane segments and their connecting loops (M1/M2, M3/M4, M5/M6) for two reasons; first, because several members of the class terminate after M6 and second, because the sites for specific inhibitors are known to be lumenally exposed. A novel procedure was used to build the models. Multiple alignments using more than 20 sequences were constructed for the regions around each membrane pair and the species with the shortest loops identified. Their canonical nature allowed modeling the 'minimal loops' from similar helix-loop-helix structures found in the Brookhaven Data Base (transmembrane segments were assumed to be helical). Thus the short loops identified the helix orientations for each membrane pair in the P-type family (a 'homology modeling' assumption borne out by the alignments). Two models were built based on these results, K-ATPase of *S. faecalis* and hog gastric HK-ATPase. Larger loops were inserted while maintaining helix orientations. Assembly of the transmembrane pairs was influenced by factors such as potential charge and hydrogen bond interactions and the apparent position of the lipid. Comparison of the models identifies potential ion binding sites and suggests a mode of inhibitor action for clinically important inhibitors of acid secretion.

W-Pos2

DIFFERENTIAL EFFECT OF BRAIN/CARDIAC BETA-SUBUNIT (β_B) ON CLONED FULL LENGTH CARDIAC ALPHA-SUBUNIT (α_{IC}) AND CARDIAC (α_{IC})-SKELETAL (α_{IS}) CHIMERIC ($\alpha_{IC/IS}$) CALCIUM CHANNELS. (Maria Laura Messi, Murali Gopalakrishnan and Osvaldo Delbono). Bowman Gray School of Medicine. Wake Forest University, Dept. of Physiology and Internal Medicine, Winston-Salem, NC 27157.

A conserved motif in α_{IS} , α_{IA} , α_{IB} and α_{IC} I-II cytoplasmic linker plays an important role in channel modulation by the β -subunit (Pragnell et al., Nature 368: 67-70, 1994). Recent studies on cardiac (α_{IC}) and cardiac (α_{IC})-skeletal (α_{IS}) chimeric calcium channel construct suggest that changes in α_{IC} amino terminus and Domain I sequences modify the interaction with beta subunit (β_B) (Delbono et al., Biophys. J. 66: A88, 1994). In this work, gating and ionic currents in *Xenopus* oocytes expressing cRNA encoding α_{IC} and $\alpha_{IC/IS}$ alone or in association with β_B , were recorded. α_{IC} and $\alpha_{IC/IS}$ share the same I-II linker. An small area of the oocytes were voltage-clamped at $V_h = -90$ mV and recorded in 10 mM Ba²⁺. Gating currents were measured in a solution containing 2 mM CoCl₂. Oocytes were permeabilized with 0.1% saponin and equilibrated with 110 mM potassium glutamate. Maximal membrane conductance (G_{max}) in cardiac (α_{IC}) was increased five fold by coexpressing β_B . G_{max} (nS/nF) were 40 ± 11 (n=17) and 207 ± 24 (n=20), in α_{IC} and α_{IC} plus β_B , respectively. G_{max} (nS/nF) in $\alpha_{IC/IS}$ alone and plus β_B were 57 ± 14 and 954 ± 66 (n=15), respectively. Beta subunit promoted 15-16 fold increase in G_{max} . Maximum gating currents (Q_{max}) in α_{IC} were barely modified by β_B coexpression (1.52 ± 0.28 and 1.64 ± 0.17 pC/nF, respectively, n=12). Gating currents were undetectable in oocytes injected with $\alpha_{IC/IS}$ alone (n=10). When the chimeric α was coinjected with β_B , Q_{max} (8.5 pC/nF) was five fold the maximum charge measured in α_{IC} plus β_B (1.66 ± 0.37 , n=12). These results suggest that, in addition to I-II linker of α , Domain I plays an important role in the interaction with beta-subunit.